ISSN 1346-7565 Acta Phytotax. Geobot. 53 (2): 143–152 (2002)

Chromosome Numbers of Japanese Asclepiadaceae

TADASHI YAMASHIRO[1*], MASATSUGU YOKOTA[2], TETSUO DENDA[2], YOICHI TATEISHI[3], JUN YOKOYAMA[4] and MASAYUKI MAKI[4]

[1]Biological Institute, Graduate School of Science, Tohoku University, Aoba, Sendai, 980-8578, Japan; [2] Faculty of Science, University of the Ryukyus, Nishihara, 903-0213, Japan; [3] Department of Science Education, Faculty of Education, University of the Ryukyus, Nishihara, 903-0213, Japan; [4]Division of Ecology and Evolutionary Biology, Graduate School of Life Sciences, Tohoku University, Aoba, Sendai, 980-8578, Japan

Somatic chromosome numbers were examined for 28 species and two varieties of seven genera in Asclepiadaceae collected in Japan. The chromosome numbers of 21 species and two varieties were newly reported here. All taxa examined had chromosome numbers of $2n = 22$ except for $2n = 44$ in *Cynanchum caudatum* var. *caudatum*, *Vincetoxicum ambiguum*, *V. sublanceolatum* var. *sublanceolatum*, *V. sublanceolatum* var. *macranthum* and $2n = 24$ for *Cynanchum boudieri*. In *Cynanchum caudatum*, chromosome number of $2n = 22$ was also found as well as $2n = 44$, indicating that intraspecific polyploidy exists in the species.

Key words: Asclepiadaceae, chromosome number, intraspecific polyploidy, Japan

The family Asclepiadaceae comprises approximately 2000 species and mainly occurs in tropical and subtropical regions through the world (Zomlefer 1994). In Japan, 36 species in nine genera are recorded, and all the species belong to the tribe Asclepiadeae (three genera) or Marsdenieae (six genera) of the subfamily Asclepiadoideae (Yamazaki 1993).

Although chromosome numbers of more than 300 species in Asclepiadaceae have been counted so far, most of previous cytological studies focused on succulent species of the tribe *Stapelieae* (Albers 1983). Excepting the tribe, relatively little information about chromosome number is available for the species of Asclepiadaceae (Albers 1983). According to previous reports on chromosome counts, $x = 11$ is the most frequently observed basic chromosome number in Asclepiadaceae and is therefore considered to be an ancestral condition (Albers 1983). In some genera, *Araujia* Bort., *Cynanchum* L., *Daemia* R. Br., *Heterostemma* Wight & Arn., *Microloma* R. Br., *Sarcostemma* R. Br., and *Vincetoxicum* Wolf, however, several basic chromosome numbers such as $x = 8$, 9, 10, 11 and 12 have been reported for some species (Albers *et al.* 1993).

In Japanese Asclepiadaceae, chromosome numbers were counted for 10 species (Yamazaki 1993). However, only three species, *Cynanchum caudatum* (Miq.) Maxim. (Nishikawa 1985), *Hoya carnosa* (L. f.) R. Br. (Nakamura 1993), *Vincetoxicum japonicum* Morr. et Decne. (Jinno 1956), have been studied for chromosome numbers using the materials collected in Japan.

The most wide-spread and distinctive cytogenetic process which has affected the evolution of

---

*Author for correspondence: Phone & Fax: 022-217-6690. E-mail: *gusuku@mail.cc.tohoku.ac.jp*

TABLE. 1. List of taxa, localities, voucher and chromosome numbers of Japanese Asclepiadaceae

Taxa	Localities	2n	voucher**
Asclepiadeae			
Cynanchum boudieri H. Lév. et Vaniot	Kagoshima Pref., Amami Isl., Yamato-son, Naon, Fukumoto logging road, alt. 440 m.	24*	Ta & YaT 45546
C. caudatum (Miq.) Maxim. var. *caudatum*	Shizuoka Pref., Iwata-gun, Misakubo-cho,. alt. ca. 1200 m.	22	YaT 3984
	Nagano Pref., Sarashina-gun, Yasaka-mura, Karakemi Moor, alt. ca. 950 m.	22	Ta & YaT 55290
	Yamanashi Pref., Minamitsuru-gun, N. E. foot of Mt. Fuji, Kitafuji Maneuver site, alt. ca. 1200 m.	44*	Ta 45367
var. *tanzawamontanum* Kigawa	Kanagawa Pref., Ashigarakami-gun, Yamakita-cho, Nishizawa – Azegamaru-yama.	22*	YaT 7581
C. liukiuense Warb.	Okinawa Pref., Ishigaki Isl., Mt. Omoto-dake.	22*	YaT 4069
C. wilfordii (Maxim.) Hemsl.	Shizuoka Pref., Inasa-gun, Mikkabi-cho, Honzaka Pass.	22*	YaT 3830
Metaplexis japonica (Thunb.) Makino	Hokkaido Pref., Shiraoi-gun, Shiraoi-cho, Hagino.	22	Yo s. n.
	Miyagi Pref., Sendai-shi, Aoba-ku, Kawauchikita, in the campus of Tohoku Univ.	22	YaT 7585
	Fukushima Pref., Sukagawa-shi, Mujinamori.	22	YaT 7586
	Shizuoka Pref., Inasa-gun, Mikkabi-cho, Honzaka Pass.	22	YaT 4184
	Nagano Pref., Kiso-gun, Hiyoshi-mura. alt. 840-980 m.	22	Ta, YaT & HC 55287
Tylophora aristolochioides Miq.	Miyagi Pref., Kakuda-shi, Mt. Tokura-yama.	22*	YaT & YaA 7580
T. floribunda Miq.	Aichi Pref., Shinshiro-shi, N. foot of Mt Kichisho-yama.	22*	YaT 3937
T. japonica Miq.	Okinawa Pref., Nakijin-son, Mt. Oppa-dake, alt. 50-120 m.	22*	YaT 3737
T. tanakae Maxim.	Okinawa Pref., Itoman-shi, Gushikawajyoshi.	22*	YaT 4003
	Okinawa Pref., Yonaguni Isl, Kubura.	22*	YaT 4198
Vincetoxicum acuminatum Decne.	Gunma Pref., Kitagunma-gun, Ikaho-cho, Mts. Haruna-san, Mt. Futatsu-dake.	22	YaT & YaA 7469
V. ambiguum Maxim.	Miyazaki Pref., Miyazaki-shi, Sadowara-cho, Uedashima.	44*	YaT & YaA 7320
V. amplexicaule Sieb. et Zucc.	Miyazaki Pref., Higashimorokata-gun, Takaoka-cho, Yoreshi.	22*	YaT & YaA 7234
	Nagasaki Pref., Fukue-shi, Mt. Oni-dake.	22*	YaT & YaA 7454
V. atratum (Bunge) Morr. et Decne.	Yamanashi Pref., Minamitsuru-gun, N. E. foot of Mt. Fuji, Kitafuji Maneuver site, alt. ca. 1200 m.	22	Ta 45370
V. austrokiusianum (Koidz.) Kitagawa	Kagoshima Pref., Kaseda-shi, Tetsusan, alt. ca. 200 m.	22*	Ta, O & YaT 45499
V. calcareum (Ohashi) Akasawa	Kochi Pref., Kami-gun, Mononobe-son, Mt. Ishidate-yama, alt. ca. 1600 m.	22*	YaT & YaA 7199
V. japonicum Morr. et Decne.	Aichi Pref., Atsumi-gun, Atsumi-cho, Irago, Hinodenomon.	22	YaT 3853
	Nagasaki Pref., Nishisonogi-gun, Sanwa-cho, Oike.	22	YaT & YaA 7452

TABLE. 1. continued

Taxa	Localities	2n	voucher**
	Hyogo Pref., Mihara-gun, Mihara-cho, Nariai reservoir.	22	YaT & YaA 7434
V. katoi (Ohwi) Kitagawa	Shizuoka Pref., Fukuroi-shi, Toyosawa.	22*	YaT & YaA 7181
	Aichi Pref., Shinshiro-shi, Mt. Kichisho-yama, alt. ca. 350 m.	22*	YaT & YaA 7421
	Chiba Pref., Inba-gun, Tomisato-cho, Nekona.	22*	YaT & YaA 7417
V. macrophyllum Sieb. et Zucc.	Nagasaki Pref., Kitatakaki-gun, Takaki-cho, Mts. Tara-dake.	22*	YaT & YaA 7457
	Gunma pref., Tone-gun, Niiharu-mura, Hoshi.	22*	M & HS s. n.
V. matsumurae (Yamazaki) H. Ohashi	Okinawa Pref., Kunigami-gun, Onna-son, Manzamo.	22*	Ta & YaT 45144
V. magnificum (Naki) Kitagawa	Iwate Pref., Kesen-gun, Sumita-cho.	22*	Taz s. n.
V. nipponicum (Matsum.) Kitagawa	Fukushima Pref., Shirakawa-shi, Nannko Lake.	22*	YaT & YaA 7571
	Hyogo Pref., Kato-gun, Yashiro-cho, Ojiike Pond.	22*	YaT & YaA 7458
V. pycnostelma Kitagawa	Aichi Pref., Shinshiro-shi, N. foot. of Mt. Kichisho-yama.	22	YaT 3911
V. sublanceolatum (Miq.) Maxim. var. *sublanceolatum*	Aichi Pref., Shinshiro-shi, N. foot. of Mt. Kichisho-yama.	44*	YaT 4183
	Shizuoka Pref., Iwata-shi, Tsurugaike Pond.	44*	YaT & YaA 7436
	Gunma Pref., Gunma-gun, Haruna-cho, Haruna Lake.	44*	YaT & YaA 7577
var. *macranthum* Maxim.	Miyagi Pref., Izumi-ku, Mt. Izumigatake, Yoshinodaira.	44*	YaT & YaA 7536
	Yamagata Pref., Nishimurayama-gun, Nishikawa-cho, Mt. Gatussan, alt. ca. 600m.	44*	YaT & YaA 7540
V. yamanakae (Ohwi et H. Ohashi) H. Ohashi	Kochi Pref., Kami-gun, Kagami-cho, Minamitenmangu.	22*	YaT & YaA 7189
	Kochi Pref., Tosashimizu-shi, along the Masuno-gawa River.	22*	YaT & YaA 7198
Marsdenieae			
Dischidia formosana Maxim.	Okinawa Pref., Senkaku Isls., Uotsuri Isl. (Cultivated in University of the Ryukyus)	22*	YaT 7593
Jasminanthes mucronata (Blanco) W. D. Stevens & P. T. Li	Okinawa Pref., Onna-son, Manzamo.	22*	Ta 51172
	Shizuoka Pref., Fukuroi-shi, Mt. Ogasa-yama, alt. 180-250 m.	22*	YaT & YaA 7217
Marsdenia tinctoria R. Br. var. *tomentosa* Masamune	Okinawa Pref., Chinen-son, Kudeken, Seifa-utaki, alt. 60-130 m.	22*	YaT & Ta 3328
M. tomentosa Morr. et Decne.	Okinawa Pref., Ogimi-son, Mt. Nekumachiji-dake.	22*	YaT 3793
	Shizuoka Pref., Fukuroi-shi, Mt. Ogasa-yama, alt. 180-250 m.	22*	YaT 4182.

* indicates new count for the species. **: Abbreviations are HC; Hokama, C., HS; Horie, S., M; Maki, M., O; Ogawa, H., Ta; Tateishi, Y., Taz; Tazawa, Y., YaT; Yamashiro, T., YaA; Yamashiro, A., Yo; Yokoyama, J.

higher plants is polyploidy (Stebbins 1971). Thus, the cytological information is one of the most essential to examine phylogeny and speciation in a certain family. To provide additional cytological information about Japanese Asclepiadaceae, we report here chromosome numbers for 28 species and two varieties of seven genera in Asclepiadaceae collected in Japan.

Materials and Methods

Twenty-eight species and two varieties belonging to seven genera were collected from a total of 50 localities in Japan (Table 1). The collected plants were cultivated in pots in the green house of University of the Ryukyus or the experimental gardens of Tohoku University.

Taxonomic treatments of the species examined follow Kitagawa (1959), Akasawa (1981), Kigawa (1989), Ohashi (1990), Yamazaki (1993), Li *et al.* (1995), and Endress & Bruyns (2000). Voucher specimens are deposited at TUS and URO.

For the observation of somatic chromosomes, root tips were pretreated in a 2mM 8-hydroxyquinoline aqueous solution for 3.5 hours at room temperature. The root tips were then fixed with a mixture of 100% ethanol and acetic acid (3:1) for 30 minutes and preserved at 5°C until the observations that followed after maceration with a mixture of 1N HCl and 45% acetic acid (2:1) at 60°C for 30 seconds and were staining in 2% aceto-orcein solution for an hour. The meristematic parts of the root-tips were squashed in 2% aceto-orcein on slide glasses and were observed using a microscope at 1500 magnification. Chromosome numbers of at least three cells for each individual were counted at somatic metaphase.

Results and Discussion

The chromosome numbers of 28 species and two varieties observed in this study are presented in Table 1 and Figs. 1 - 31. Of these taxa, the chromosome number of 21 species and the two varieties were newly counted in this study (Table 1).

All of 30 taxa investigated in this study had chromosome number of $2n = 22$ except for *Cynanchum boudieri* H. Lév. et Vaniot ($2n = 24$), *C. caudatum* var. *caudatum* ($2n = 22, 44$), *Vincetoxicum ambiguum* Maxim. ($2n = 44$), *V. sublanceolatum* (Miq.) Maxim. var. *sublanceolatum* (Miq.) Maxim. ($2n = 44$), and *V. sublanceolatum* (Miq.) Maxim. var. *macranthum* Maxim. ($2n = 44$). The chromosome numbers of $2n = 22$ and 24 represent diploid of $x = 11$ and $x = 12$, respectively, while $2n = 44$ represents tetraploid of $x = 11$. In *Cynanchum caudatum* var. *caudatum*, tetraploid was found only in the individual collected on Mt. Fuji, while diploids were found in other two localities. At this point, it is uncertain that the tetraploids observed in this study are autotetraploid or allotetraploid. Genetic studies aided with molecular makers are desirable to analyze the origin of these tetraploid taxa.

Chromosome counts in the present study are consistent with previous reports of $2n = 22$ for *C. caudatum* (Nishikawa 1985), *V. acuminatum* Decne. (Probatova & Sokolovskaya 1983), *V. atratum* (Bunge) Morr. et Decne. (Ge et al. 1987, Probatova & Sokolovskaya 1990), *V. japonicum* (Jinno 1956) and *V. pycnostelma* Kitagawa (Ge *et al.* 1987). By contrast, our results disagreed with previous reports for some taxa. We counted the chromosome number of $2n = 22$ for *Metaplexis japonica* (Thunb.) Makino from four localities in Japan, while $2n = 24$ has been reported for the same species from Russia and China (Skolovskaya 1966, Skolovskaya & Probatova 1986, Ge *et al.* 1988). Similarly, although chromosome numbers of $2n = 16$ and $2n = 24$ have been reported in *V. amplexicaule* Sieb. et Zucc. from Korea (Lee 1970) and *V. japonicum* from Russia (Gieszczykowna 1934), respectively, we observed $2n = 22$ for these two species in Japan. Japanese populations of these species may be cytologically differentiated from the continental one. Otherwise, it is possible to explain these disagreements of the chromosome numbers by misidentification of the materials or miscounting of

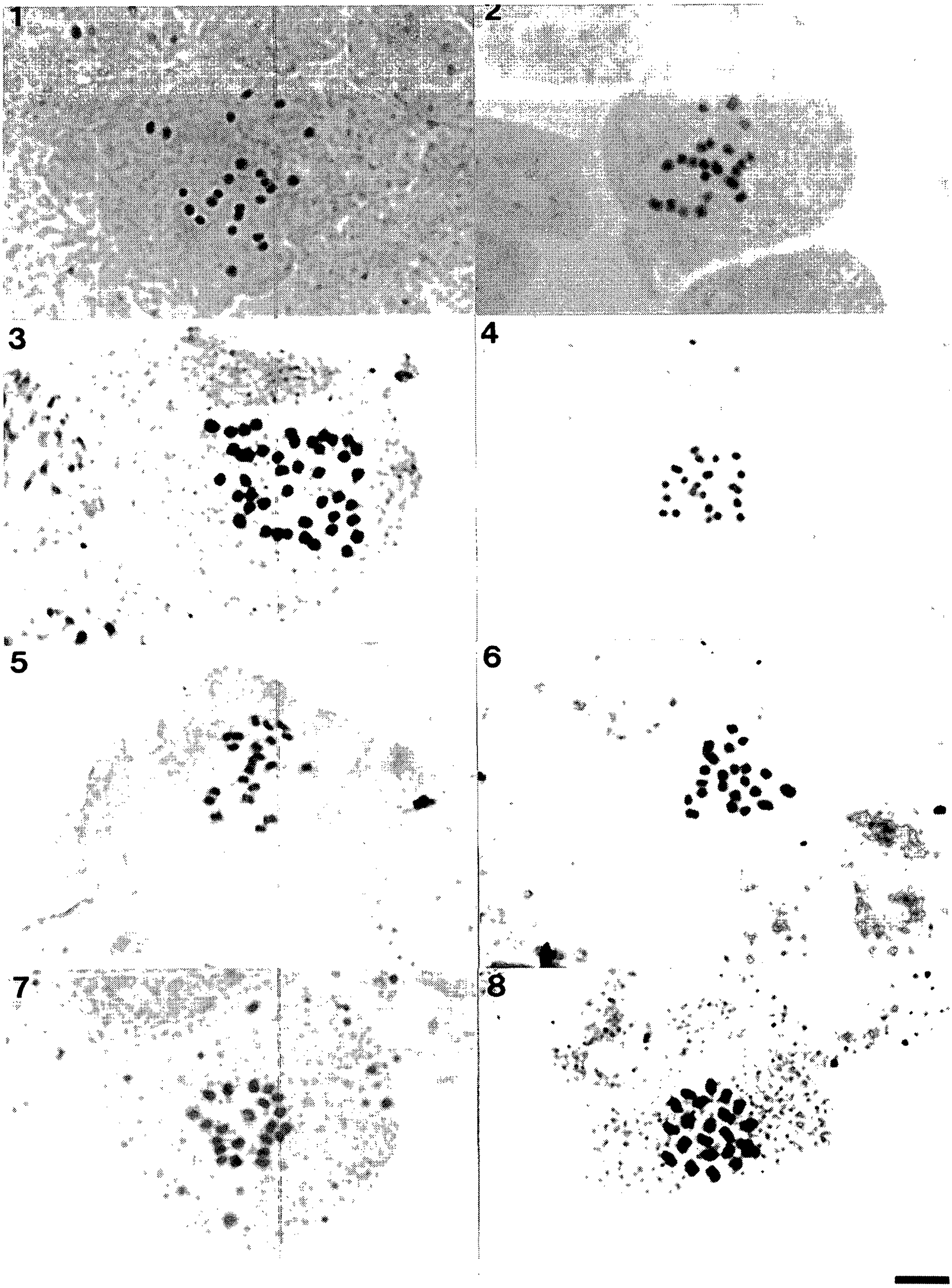

FIG. 1-8. Microphotographs of somatic chromosomes at metaphase of Japanese Asclepiadaceae. 1. *Cynanchum boudieri* (2*n* = 24). 2. *C. caudatum* (2*n* = 22). 3. *C. caudatum* (2*n* = 44). 4. *C. caudatum* var. *tanzawamontanum* (2*n* = 22). 5. *C. liukiuense* (2*n* = 22). 6. *C. wilfordii* (2*n* = 22). 7. *Dischidia formosana* (2*n* = 22). 8. *Jasminanthes mucronata* (2*n* = 22). Scale bar represents 4 μm.

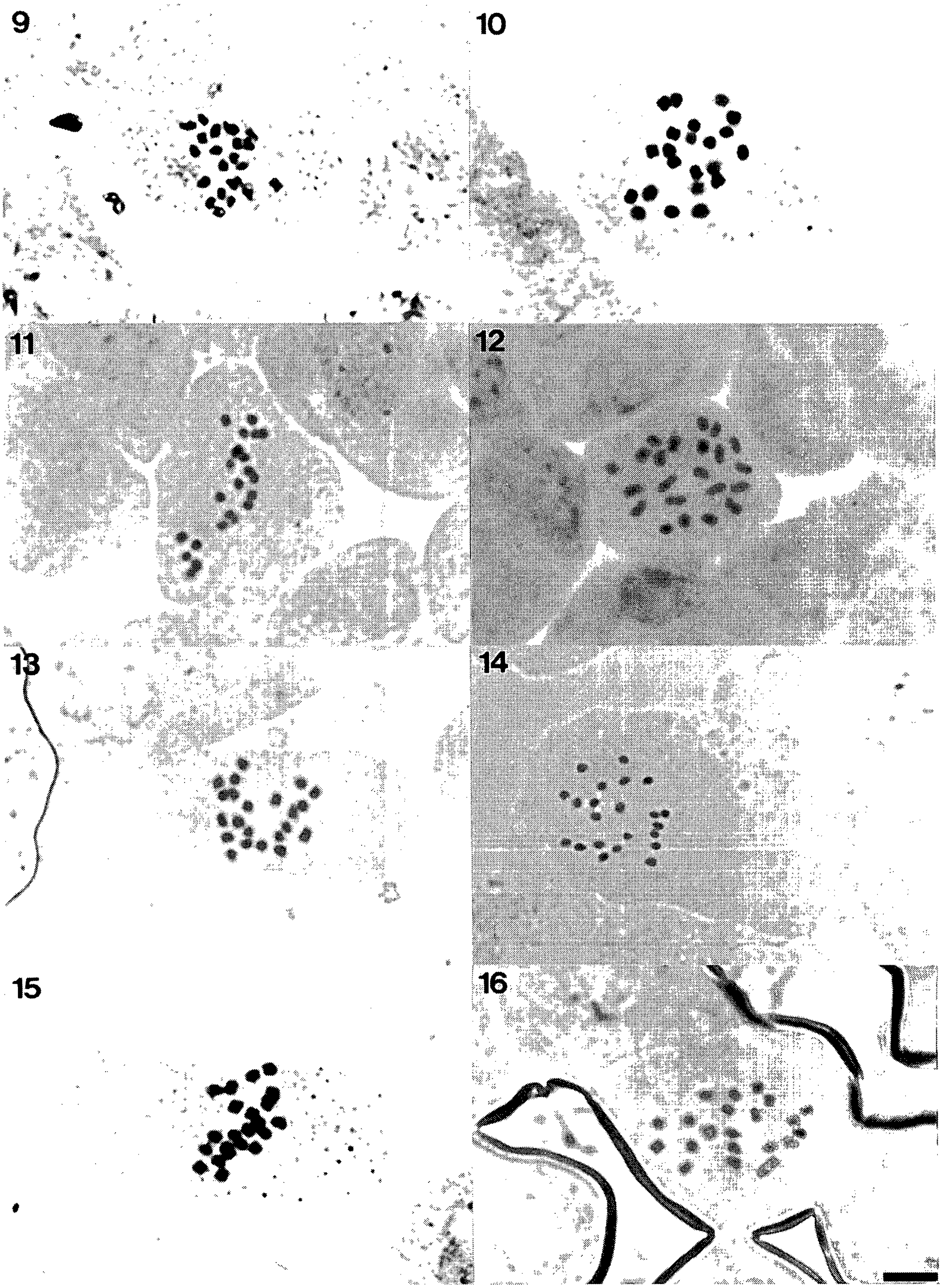

FIG. 9-16. Microphotographs of somatic chromosomes at metaphase of Japanese Asclepiadaceae. 9. *Marsdenia tinctoria* var. *tomentosa* ($2n = 22$). 10. *M. tomentosa* ($2n = 22$). 11. *Metaplexis japonica* ($2n = 22$). 12. *Tylophora aristolochioides* ($2n = 22$). 13. *T. floribunda* ($2n = 22$). 14. *T. japonica* ($2n = 22$). 15. *T. tanakae* ($2n = 22$). 16. *Vincetoxicum acuminatum* ($2n = 22$). Scale bar represents 4 μm.

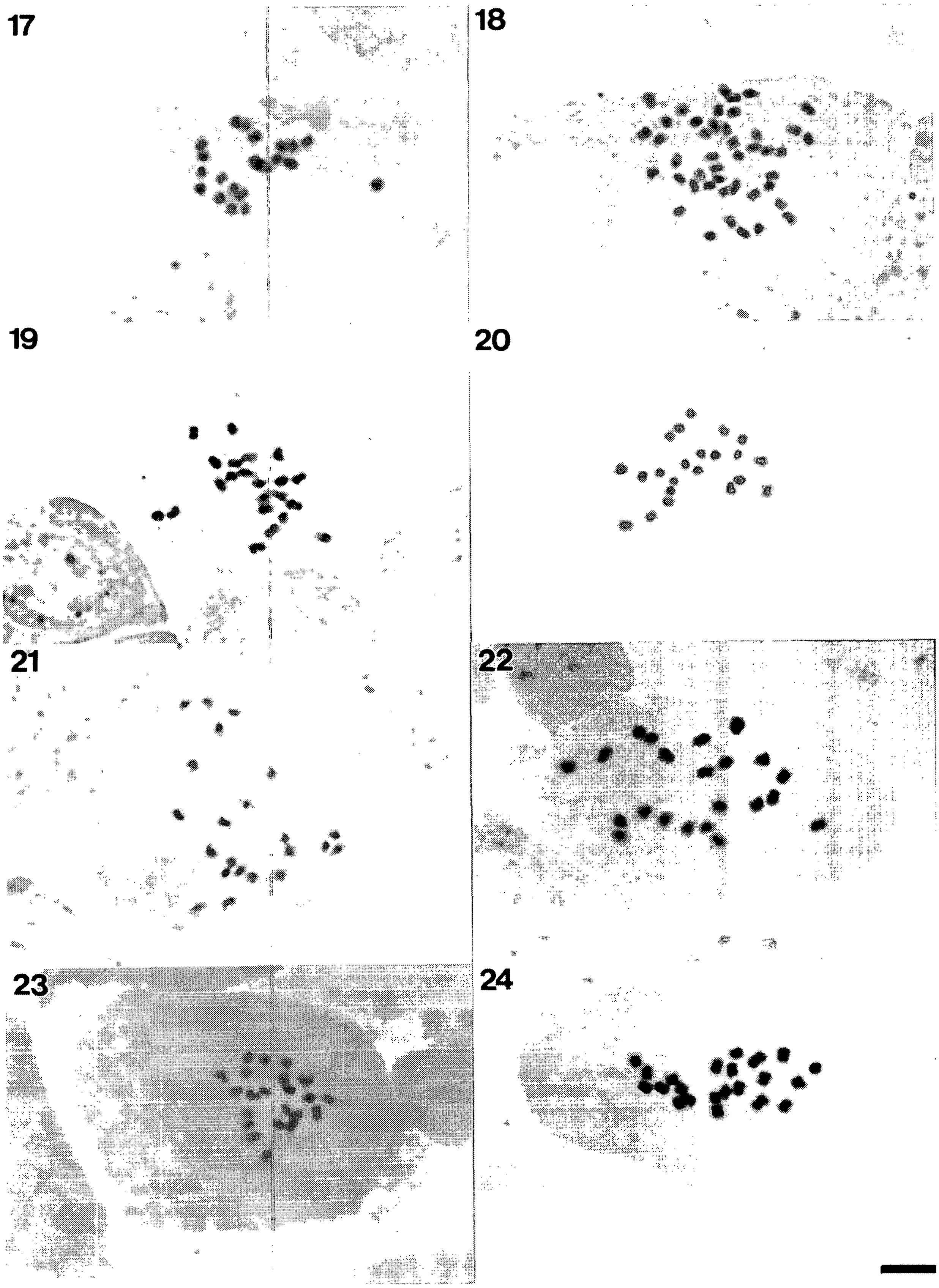

FIG. 17-24. Microphotographs of somatic chromosomes at metaphase of Japanese Asclepiadaceae. 17. *Vincetoxicum calcareum* (2*n* = 22). 18. *V. ambiguum* (2*n* = 44). 19. *V. amplexicaule* (2*n* = 22). 20. *V. atratum* (2*n* = 22). 21. *V. austrokiusianum* (2*n* = 22). 22. *V. japonicum* (2*n* = 22). 23. *V. katoi* (2*n* = 22). 24. *V. macrophyllum* (2*n* = 22). Scale bar represents 4 μm.

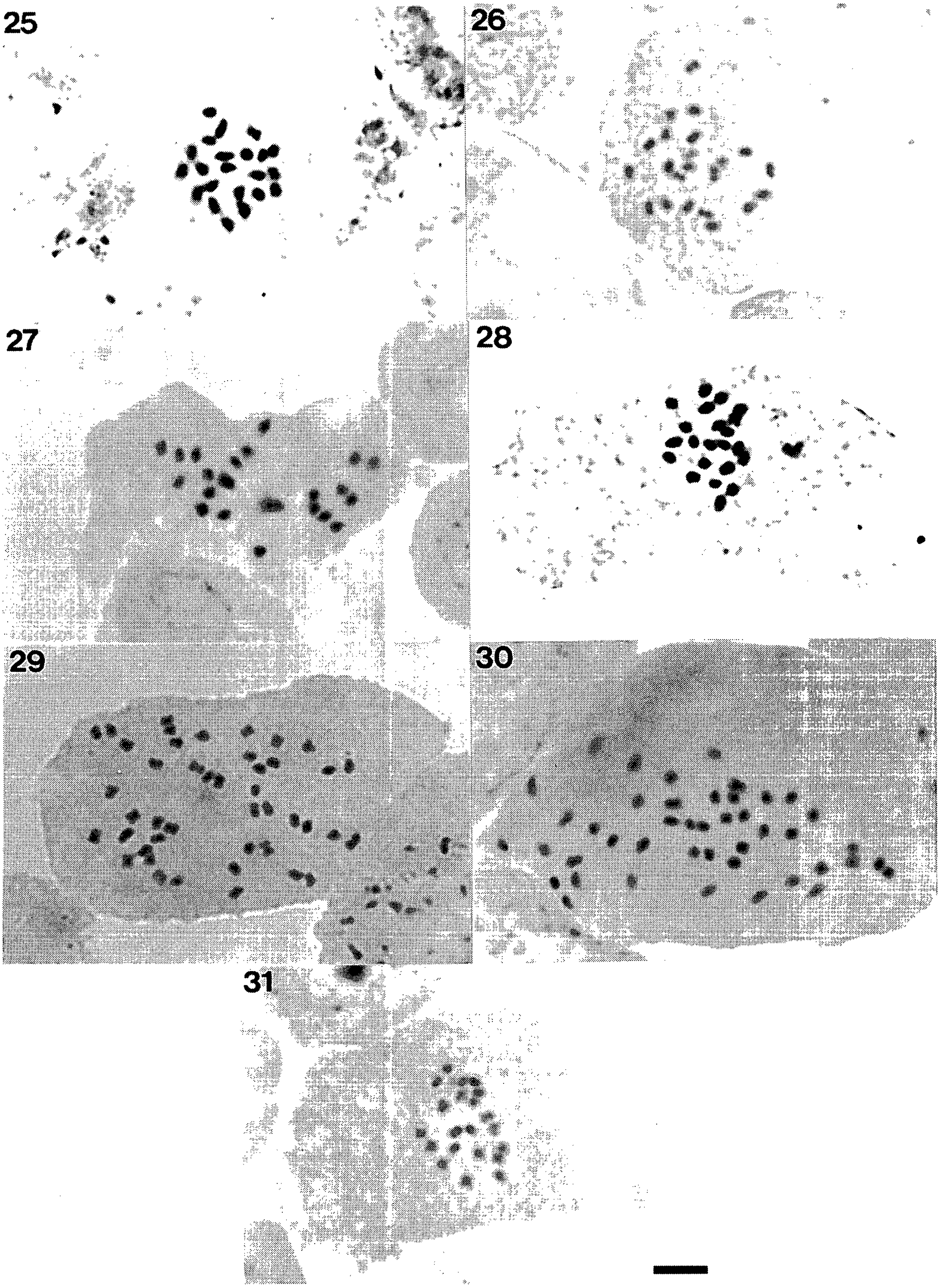

FIG. 25-31. Microphotographs of somatic chromosomes at metaphase of Japanese Asclepiadaceae. 25. *Vincetoxicum matsumurae* ($2n = 22$). 26. *V. magnificum* ($2n = 22$). 27. *V. nipponicum* ($2n = 22$). 28. *V. pycnostelma* ($2n = 22$). 29. *V. sublanceolatum* var. *sublanceolatum* ($2n = 44$). 30. *V. sublanceolatum* var. *macranthum* ($2n = 44$). 31. *V. yamanakae* ($2n = 22$). Scale bar represents 4 μm.

chromosome numbers. Further cytological studies on continental materials are needed.

The chromosome number of *Cynanchum boudieri* was counted to be $2n = 24$. Although *C. boudieri* is also distributed in Taiwan and south China, the chromosome number of this species has not been investigated in these areas (Li *et al.* 1995). In the genus *Cynanchum*, $2n = 24$ has been reported for two species, *C. sibiricum* Willd. and *C. virens* Dietr. (Rostovtseva 1977, Albers *et al.* 1993), and all other species have 22 or 44 chromosomes. The basic chromosome numbers of $x = 12$ is also found among several genera such as *Metaplexis* R. Br., *Pergularia* L. and *Heterostemma* (Skolovskaya 1966, Skolovskaya & Probatova 1986, Ge *et al.* 1988, Albers *et al.* 1993). Considering that these genera are taxonomically distinct from each other and that the basic chromosome number of $x = 11$ may be an ancestral condition in Asclepiadaceae, evolutionary changes from x=11 to x=12 seem to have occurred recurrently in this family. To test this hypothesis, phylogenetic studies using molecular information are needed.

The authors thank Dr. H. Ohashi for useful comments, and Dr. N. Tanaka, Messers. N. Inagaki, Y. Koukami, T. Minamitani, T. Orime and A. Yamashiro for collecting materials. Our thanks are also due to Mr. K. Sato for cultivation of material plants.

References

Akasawa, Y. 1981. Notulae ad plantas Sikokianae XI. Bull. Kochi Women's Univ. Ser. Nat. Sic. 29: 1-7(in Japanese).

Albers, F. 1983. Cytotaxonomic studies in African Asclepiadaceae. Bothalia 14: 795-798.

——, S. Liede & U. Meve. 1993 Deviating chromosome numbers in Asclepiadaceae. Nord. J. Bot. 13: 37-39.

Endress, M. E. & P. V. Bruyns. 2000. A revised classification of the Apocynaceae *s. l.* Bot. Rev. 66: 1-56.

Ge, C. J., Y. K. Li, Y. Zhou & P. S. Hsu. 1987. Observations on the chromosome numbers of medical plants of Shandong Province (III). Acta Bot. Yunn. 9: 116-118 (in Chinese with English summary).

——, ——, P. Wan, Y. X. Li & F. H. Jiang. 1988. Observations on the chromosome numbers of medicinal plants from Shandong Province (V). J. Shangdong Coll. Traditional Chin. Med. 12: 55-57.

Gieszczykowna, Z. 1934. Rozwoj pylku i liczba chromozomow u niektorych gatunkow rodzaju *Vincetoxicum*. Acta Soc. Bot. Polon. 2: 393-397 (in Russian).

Jinno, T. 1956. On the relation between the chromosome numbers and the flora growing in the coast of the inland sea in Japan. Jap. J. Genet. 31:147-150.

Kigawa, S. 1989. On the two new varieties and a new form of the plants from Kanagawa Prefecture. Bull. Kanagawa Pref. Mus. 18: 11- 22 (in Japanese).

Kitagawa, M. 1959. Notulae fractae ob floram Asiae Orientalis (12). J. Jap. Bot. 34: 361-366 (in Japanese).

Lee, Y. N. 1970. Chromosome numbers of flowering plants in Korea (3). J. Korean Res. Inst. Better Living 5: 127-129.

Li, P. T., M. G. Gilbert & W. G. Stevens. 1995. Asclepiadaceae. *In*: Wu, Z. Y. & P. H. Raven (eds.), Flora of China 16: 189-270. Missori Botanical Garden, St. Louis.

Nakamura, T. 1993. Speciation of *Hoya carnosa* (Asclepiadaceae). La KromosomoII71-72: 2479-2489 (in Japanese with English summary).

Nishikawa, T. 1985. Chromosome counts of flowering plants of Hokkaido (8). J. Hokkaido Univ. Educ., Sect. 2B. 35: 97-111.

Ohashi, H. 1990. New combinations in Japanese *Vincetoxicum*. J. Jap. Bot. 65: 277-278 (in Japanese).

Probatova, N. S. & A. P. Sokolovskaya. 1983. New chromosome numbers for vascular plants from the islands of Peter the Great Bay (Primorye Territory). Bot. Zurn. 68: 1655-1662 (in Russian).

—— & ——. 1990. Chromosome numbers in some representatives of the families Asclepiadaceae, Asteraceae, Boraginaceae, Chenopodiaceae, Lamiaceae, Oleaceae. Onagraceae, Scrophulariaceae, Solanaceae, Urticaceae from the Soviet Far East. Bot. Zurn. 75: 1619-1622 (in Russian).

Rostovtseva, T. S. 1977. Chromosome numbers in some plant species from the south of Siberia.II. Bot. Zurn. 62: 1034-1042 (in Russian).

Sokolovskaya, A. P. 1966. Geograficheskoe rasprostranenie poliploidnykh vidov rasteniy. (Issledovanie flory Primoskogo kraya). Vestnik Leningr. Univ. 1966, Ser. Biol. 3: 92-106 (in Russian).

—— & N. S. Probatova. 1986. Chromosome numbers and distribution of some anthropophytes in Primorye territory and Amur River Valley. Vestn. Leningradsk.

Univ. Ser. 3, Biol. 2: 57-63 (in Russian).

Stebbins, G. L. 1971. Chromosomal evolution in higher plants. Arnold, London.

Yamazaki, T. 1993. Asclepiadaceae. *In*: Iwatsuki, K., T. Yamazaki, D. E. Boufford & H. Ohba (eds.), Flora of Japan 3(a): 168-182. Kodansha, Tokyo.

Zomlefer, W. B. 1994. Guide to flowering plant families. pp. 239-244. The University of North Carolina Press, Chapel Hill.

Received March 12, 2002; accepted May 23, 2002